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ORIGINAL RESEARCH ARTICLE

Evidence of household transfer of ESBL-/pAmpC-producing Enterobacteriaceae between humans and dogs – a pilot study

Oskar Ljungquist, MD[1]*, Ditte Ljungquist, DVM[2], Mattias Myrenås, MSc[3], Cecilia Rydén, MD, PhD, Associate Professor[1], Maria Finn, MLS[3] and Björn Bengtsson, DVM, PhD, Associate Professor[3]

[1]Department of Infectious Disease, Helsingborg's Hospital, Helsingborg, Sweden; [2]Evidensia Small Animal Hospital, Helsingborg, Sweden; [3]Swedish National Veterinary Institute, Uppsala, Sweden

Background: Extended-spectrum cephalosporin-resistant Enterobacteriaceae (ESCRE) are an increasing healthcare problem in both human and veterinary medicine. The spread of ESCRE is complex with multiple reservoirs and different transmission routes. The aim of this study was to investigate if ESCRE carriage in dogs is more prevalent in households with a known human carrier, compared to households where humans are known to be negative for ESCRE. Identical ESCRE strains in humans and dogs of the same household would suggest a possible spread between humans and dogs.

Methods: Twenty-two dog owners with a positive rectal culture for ESCRE each collected a rectal sample from their dog. In addition, a control group of 29 healthy dog owners with a documented negative rectal culture for ESCRE each sampled their household dog. Samples were cultivated for ESCRE using selective methods. In households where both humans and dogs carried ESCRE, isolates were further analysed for antimicrobial susceptibility by disc diffusion or microdilution and for genotype and genetic relatedness using molecular methods.

Results: In 2 of 22 households studied, identical ESCRE strains with respect to bacterial species, antibiogram, genotype, and MLVA type were found in humans and dogs. The ESCRE found in the two households were ESBL-producing *E. coli* with the resistance gene $bla_{\text{CTX-M-27}}$ and AmpC-producing *E. coli* with $bla_{\text{CMY-2}}$, $bla_{\text{TEM-1}}$. ESCRE were not found in dogs in the control group.

Conclusions: In households where humans are carrying ESCRE, identical strains were to a limited extent found also in household dogs, indicating a transfer between humans and dogs. In contrast, ESCRE were not found in dogs in households without human carriers.

Keywords: *animal*; *human*; *antimicrobial resistance*; *Escherichia coli*; *cephalosporin*

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*Correspondence to: Oskar Ljungquist, Department of Infectious Disease, Helsingborg's Hospital, SE-251 87 Helsingborg, Sweden, Email: oskarljungquist@hotmail.com

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Enterobacteriaceae resistant to extended-spectrum cephalosporins (ESC) by production of extended-spectrum beta-lactamase (ESBL) or plasmid-mediated AmpC beta-lactamase (pAmpC) have emerged as a serious healthcare problem worldwide both in human and veterinary medicine (1, 2). Extended-spectrum cephalosporin-resistant Enterobacteriaceae (ESCRE) are found in humans and animals and in the environment; therefore, the spread of such bacteria is complex with multiple reservoirs and different transmission routes (1). Moreover, Enterobacteriaceae, including ESCRE, can be part of the normal gut flora of healthy humans and animals, including dogs (3).

The prevalence of ESCRE in Swedish health care is low compared to many other countries, but the number of total human cases with such bacteria has increased by 9–33% annually since 2007, including asymptomatic carriers and ESCRE infections (4). Recent studies found a 5% prevalence of asymptomatic faecal ESCRE carriage in healthy Swedes (5) and a 3% prevalence of ESBL-producing *E. coli* in preschool children (6). Similar levels in healthy humans are reported from other European countries (7–10). In other parts of the world, notably Southeast Asia, the Eastern Mediterranean, and the Western Pacific, the prevalence of ESCRE in healthy humans is much higher (10). This is reflected in a high

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incidence of ESCRE carriage in travellers returning from high-prevalence areas to Sweden (11) or to other low-prevalence countries (7, 8).

ESCRE are found in food-producing animals worldwide and in meat thereof, potentially spreading from animals to humans through the food chain (1, 12). ESCRE are also found in healthy and diseased companion animals such as dogs (1, 13–19), and the potential zoonotic risk of this has been emphasized (20). The prevalence of ESCRE in dogs in Sweden is largely unknown, but in a recent study screening clinical urinary tract samples from dogs, ESCRE was found in less than 0.1% of 1,042 samples (21), and in a screening of 84 healthy dogs in 2012 only one carried ESCRE (22). Thus, clinical cases with ESCRE in dogs in Sweden are rare, but the number of confirmed cases has increased in the last years (4, 22, 23).

Risk factors for humans acquiring ESCRE are, for example, foreign travel, recent antibiotic treatment, and having/living with a family member treated for urinary tract infection caused by an ESCRE (24). Pet ownership is also reported to be a risk factor for ESCRE carriage (25). As for humans, dogs recently treated with antibiotics or dogs being kept in shelters or at breeders were identified to be at risk for being colonized with ESCRE (14, 15).

Humans and dogs are known to be able to share identical strains of Enterobacteriaceae, suggesting spread of bacteria between humans and dogs (26–29). Moreover, the same plasmids carrying genes coding for ESC resistance are found in ESCRE from humans and dogs and, thus, household dogs are potential reservoirs and may expose humans of the same household to ESCRE (13, 30, 31). However, little is known about the extent to which ESCRE are spread between humans and dogs in the same household.

The aim of this study was to investigate if ESCRE carriage in dogs is more prevalent in households with a known human carrier, compared to households where humans are known to be negative for ESCRE. Identical ESCRE strains in humans and dogs of the same household would suggest a possible spread between humans and dogs.

Materials and methods

Study group

The study group consisted of persons known to carry ESCRE in the intestinal flora. Inclusion criteria for entering the study group were positive for selective ESCRE screening from rectal/faecal swabs in the recent 3 months and at least one dog in the household. Persons in the study group were selected from patients receiving health care in the region of Skåne with a total population of 1,286,584 people (32). In an 18-month period between March 2014 and August 2015, 498 patients were positive for ESCRE in a selective cultivation of faeces at the Department of Clinical Microbiology, Skånes University Hospital. The samples had been taken from patients by caretakers all over Region Skåne, including primary, secondary, and tertiary health centres. Many of the patients were screened for ESCRE because of recent (within 6 months) hospitalization abroad. In Sweden, all patients who have received in-treatment health care abroad are screened for ESCRE by cultivation of faeces. In other patients, ESCRE were found in other cultivations, for example, urine sample, followed by a confirming cultivation from faeces.

All 498 patients with a positive selective ESCRE screening from rectal/faecal swabs were considered for inclusion in the study. Patients with ongoing prophylactic or therapeutic antibiotic treatment were excluded, as well as newborns receiving neonatal care. Patients considered for entering the study were contacted by telephone and asked for dog ownership, and if they would agree to participate in the study, collect a sample from their dog. Many patients could not be reached, for example, immigrants without contact information in the medical records.

After reviewing the medical chart and after the telephonic interview, 22 persons were enrolled in the study group. All were regarded healthy carriers of ESCRE in the intestinal tract, that is, no clinical infection with ESCRE was identified or suspected.

Enrolled persons were sent further information about the study and a formulary for informed consent to be returned to the investigator. In addition, they were sent materials and instructions on how to collect a rectal swab from one household dog. The rectal swab was sent by mail to the National Veterinary Institute (SVA), Uppsala, Sweden, for selective cultivation for ESCRE. In order to justify a temporal correlation for possible transfer of strains, the time between documented ESCRE carriage in a person until cultivation of the household dog was less than 3 months. If the dog was positive for ESCRE, other family members as well as other dogs of the household were sampled. Of the 22 persons enrolled in the study group, 20 returned the informed consent and provided rectal swabs from dogs. In addition, two persons from the control group (see below) that were positive for ESCRE on selective cultivation of a rectal swab were included in the study group.

A supplementary interview with questions regarding possible bacterial transmission between humans and dogs was conducted in households, where both dog and human were positive for ESCRE. In these households, four additional persons and two additional dogs were tested for ESCRE as described above.

Control group

The control group consisted of 31 healthy dog owners recruited from the area of Helsingborg and Uppsala.

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None were treated with antibiotics within 2 weeks prior to sampling. Persons in the control group were sent the same information and instructions on how to sample one household dog as persons in the study group. In addition, they were instructed to collect a rectal swab from themselves. This sample was sent by mail to the Department of Clinical Microbiology, Skånes University Hospital, for selective cultivation for ESCRE. Persons in the control group also provided written consent to participate in the study. Two of the persons in the control group were positive for ESCRE on selective cultivation and were transferred to the study group.

Ethical approval

Ethical approval for the study was obtained from the regional ethical review board in Lund.

Selective cultivation for ESCRE

Samples from humans

Samples from humans were cultivated for ESCRE at Department of Clinical Microbiology, Skånes University Hospital, according to the routines of the hospital. Briefly, the sample material was plated on URI-Select four agar plates with vancomycin (BioRad) complemented with two antimicrobial susceptibility discs containing ceftazidime (10 μg/mL, Oxoid) and meropenem (10 μg/mL, Oxoid), respectively, and incubated at 37°C overnight. Sample material was also plated on the chromogenic agar plate ChromID ESBL (BioMerieux) and incubated as above. Colonies of presumptive ESCRE were subcultivated on horse blood agar (HBA) and typed to bacterial species using matrix-assisted laser desorption/ionization time of flight (MALDI-TOF).

Samples from dogs

Samples from dogs were cultivated at the National Veterinary Institute. Briefly, the sample material was plated on MacConkey agar plates supplemented with cefotaxime (1 mg/mL) and incubated at 37°C overnight. In parallel, the material was enriched in liquid MacConkey broth supplemented with cefotaxime (1 mg/L) and incubated as above. If no growth was recorded on the plates, 100 μL from the enrichment was plated on MacConkey agar plate supplemented with cefotaxime (1 mg/L) and incubated at 37°C overnight. Colonies of presumptive ESCRE were subcultivated on HBA and typed to bacterial species using MALDI-TOF.

Antimicrobial susceptibility testing

Isolates from humans

Presumptive ESCRE from humans were confirmed as ESBL or pAmpC producers by susceptibility testing. Briefly, isolates were grown on Mueller–Hinton agar plates complemented with the cefpodoxime ESBL ID disc set (Mast Group Ltd). Isolates were also tested for susceptibility to imipenem, meropenem, gentamicin, tobramycin, amikacin, trimethoprim–sulfamethoxazole, tigecycline, cefotaxime, ceftazidime, and ciprofloxacin by disc diffusion using antibiotic-impregnated discs (Oxoid). Inhibition zone diameters were interpreted using the NordicAST (Nordic Committee on Antimicrobial Susceptibility Testing) breakpoints (www.nordicast.org). In addition, in households with dogs carrying ESCRE, human isolates were also tested for susceptibility by determination of minimum inhibitory concentration (MIC) by microdilution using Sensititre EUVSEC2 plates (Trek Diagnostic System Ltd) according to the standards of the Clinical and Laboratory Standards Institute (33). Antimicrobials tested were: cefotaxime, cefotaxime/clavulanic acid, ceftazidime, ceftazidime/clavulanic acid, cefepime, cefoxitin, temocillin, ertapenem, imipenem, and meropenem. MICs were interpreted using EUCAST (The European Committee on Antimicrobial Susceptibility Testing) epidemiological cutoff values (ECOFFs) (www.eucast.org/).

Isolates from dogs

Presumptive ESRCE from dogs were phenotypically confirmed as ESBL or pAmpC producers by determination of MIC by broth microdilution using EUVSEC2 plates as above.

Genotyping and epidemiological typing of presumptive ESCRE

In households where ESCRE were found in both persons and dogs, the isolates were further characterised genotypically by PCR for specific resistance genes and by multilocus variable number of tandem repeats analysis (MLVA) to determine the relatedness between isolates from dogs and humans.

DNA extraction

Total DNA extraction was performed by suspending 1 μL of colony material in 200 μL of Tris–EDTA (pH 7.5). The suspension was heated to 95°C for 15 min and centrifuged for 3 min at 20,000 × *g*. The supernatant was isolated and kept at −20°C until analysis.

Characterisation of resistance genes

ESC-resistant isolates were characterized by PCR targeting the bla_{SHV}, bla_{OXA}, bla_{TEM}, $bla_{CTX\text{-}M}$ group and pAmpC variants (MOX-1, MOX-2, CMY-1 to CMY-11, LAT-1 to LAT-4, BIL-1, DHA-1, DHA-2, ACC, MIR-1T, ACT-1, FOX1 to FOX5b) (34–36). The PCR amplicons were sequenced with in-house variants of published primers to determine the specific resistance gene variants (37–40).

MLVA

MLVA was performed according to Lobersli et al. (41). Ten previously described MLVA targets were analysed. Repeats were amplified using PCR and multiple fluorolabelled primers. PCR products were separated by capillary electrophoresis in an ABI PRISM 3100 Genetic

Analyzer (Applied Biosystems). We divided the multiplex PCR reaction M2 into two (42), separating the CVN004 and CVN007 from the CVN001 and CV015 primers, running them as two separate multiplex PCR reactions.

Each peak was normalized and identified according to colour and size using the Peak Scanner Software 2 (Applied Biosystems). An allele number was assigned based on the fragment size.

Results

Twenty-two persons confirmed to carry ESCRE from 22 households were included in the study group (Table 1). Of these, 20 carried ESBL-producing *Escherichia coli*. One of these persons also carried ESBL-producing *Klebsiella pneumonia*. Two persons carried pAmpC-producing *E. coli* and ESBL-producing *K. pneumonia*. The age of the persons ranged from 1 to 82 years. Of the 22 households with persons carrying ESCRE, 2 households (9%) had dogs that tested positive for ESCRE (Table 1). In each of these households, two additional persons were screened for ESCRE but all four were negative (Table 1). In the control group, none of the 29 dog owners or their dogs were positive for ESCRE.

Patient number 1 of household number 1

Patient 1, a 1-year-old child, belonged to a family of two adults, the child, and two dogs. All were screened for ESCRE. The child and one of the dogs carried the same ESBL-producing *E. coli* strain as determined by MLVA. The other family members, both adults, and the second dog had negative cultures. The antibiogram showed that both *E. coli* strains were resistant to cefotaxime, ceftazidime, and cefepime. The β-lactamase gene $bla_{\text{CTX-M-27}}$ was found in both isolates.

Table 1. Results of selective cultivation for ESCRE of faecal/rectal swabs from 26 humans and 24 dogs from 22 households in the study group

	Humans				Dogs		
Household	Human*	Birth year	Species/type	Genes	Dog	Species/type	Genes
1	a	2013	*E. coli* ESBL	$bla_{\text{CTX-M-27}}$	a	*E. coli* ESBL	$bla_{\text{CTX-M-27}}$
	b	1981	Not found		b	Not found	
	c	1983	Not found				
2	a	2013	*E. coli* pAmpC	$bla_{\text{CMY-2}}$, $bla_{\text{TEM-1}}$	a	*E. coli* pAmpC	$bla_{\text{CMY-2}}$, $bla_{\text{TEM-1}}$
	b	2009	Not found		b	*E. coli* pAmpC	$bla_{\text{CMY-2}}$, $bla_{\text{TEM-1}}$
	c	1976	Not found				
3	a	1942	*E. coli* ESBL	Not determined	a	Not found	
4	a	1962	*E. coli* ESBL	Not determined	a	Not found	
5	a	1988	*E. coli* ESBL	Not determined	a	Not found	
6	a	1992	*E. coli* ESBL	Not determined	a	Not found	
7	a	1973	*E. coli* ESBL	Not determined	a	Not found	
8	a	1944	*E. coli* ESBL	Not determined	a	Not found	
9	a	2013	*E. coli* ESBL	Not determined	a	Not found	
10	a	1943	*E. coli* ESBL	Not determined	a	Not found	
11	a	1948	*E. coli* ESBL	Not determined	a	Not found	
12	a	1993	*E. coli* ESBL	Not determined	a	Not found	
13	a	1958	*E. coli* ESBL	Not determined	a	Not found	
14	a	1947	*E. coli* ESBL	Not determined	a	Not found	
15	a	1974	*E. coli* ESBL	Not determined	a	Not found	
16	a	1954	*E. coli* ESBL	Not determined	a	Not found	
17	a	1932	*E. coli* ESBL	Not determined	a	Not found	
18	a	1966	*E. coli* ESBL	Not determined	a	Not found	
19	a	1939	*K. pneumonia* ESBL	Not determined	a	Not found	
20	a	1955	*E. coli* ESBL/*K. pneumonia* ESBL	Not determined	a	Not found	
21	a	1943	*E. coli* ESBL	Not determined	a	Not found	
22	a	1959	*E. coli* ESBL	Not determined	a	Not found	

In two households, four additional persons were tested. *'a' signifies the person initially enrolled in the study group, 'b and c' signify additional persons or dogs tested for ESCRE in a household.

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Patient number 2 of household number 2

Patient 2, a 2-year-old child, belonged to a family of two adults, the child, an older sibling aged 5, and two dogs. Three humans and two dogs were screened for ESCRE. One adult declined sampling. The 2-year-old child and both dogs were found to have faecal flora containing pAmpC-producing *E. coli*, whereas the other family members had negative cultures for ESCRE. The pAmpC-producing *E. coli* isolated from the child and from one of the dogs were determined to be identical by MLVA. The other dog had a similar but not identical pAmpC-producing *E coli* as determined by MLVA. The *E. coli* strains were resistant to cefotaxime, ceftazidime, cefepime, and cefoxitin. The β-lactamase genes $bla_{\text{TEM-1}}$ and $bla_{\text{CMY-2}}$ were found in all three isolates.

In interviews made with the adults in households 1 and 2, the adults reported that they had witnessed sharing of food and cutlery between toddlers and dogs.

Discussion

In this study, identical ESCRE strains with respect to bacterial species, antibiogram, genotype, and MLVA type were found in humans and dogs in 2 of the 22 households studied. This indicates that household transmission of ESCRE between humans and dogs occurs. Similar studies investigating household spread of non-ESBL-/pAmpC-producing *E coli* show that dogs and humans share identical clones (26–29, 43), but to our knowledge, this is the first report describing sharing of identical ESCRE strains between humans and dogs of the same household.

The potential risk of sharing ESCRE strains between dogs and humans within a household depends on the extent of such sharing. In our study, sharing occurred in about 10% of the households. This is of the same magnitude as in a study where 10% of 60 dog–human pairs shared *E. coli* of similar PFGE types (43). A higher extent of sharing was found in a longitudinal study, where the same AFLP types of *E. coli* were found in humans and dogs on at least one occasion over a 6-month period in four of eight studied households (29). Similarly, a higher extent of sharing was found in a study of 48 households, where the same *E. coli* clones were found in 17% of pet–human pairs (28). The extent of sharing was, however, higher (31%) between human pairs in that study. In agreement, in another study sharing of ESBL-producing *E. coli* and *K. pneumoniae* between humans of the same household was 23 and 25%, respectively (44). These latter studies indicate that humans carrying ESCRE probably are more likely to share the bacteria with other household members than with dogs.

This pilot study could not conclude on the initial transmission route of ESCRE, whether from humans to dogs or vice versa. However, since dogs were negative for ESCRE in the majority of households in the study group, the person carrying ESCRE most likely contracted the bacteria from another source than the household dog. Persons in the study group predominantly carried ESBL-producing *E. coli* and only one carried a pAmpC-producing strain. This is in accordance with the proportions of ESCRE types notified in human health care in Sweden, where the vast majority of isolates are ESBL producers and about 5% pAmpC producers (45). Interestingly, *E. coli* with the resistance gene $bla_{\text{CTX-M-27}}$, which was found in humans and dogs in household 1, has hitherto been documented only five times in dogs in Sweden comprising 7% of the 74 confirmed ESCRE isolates in dogs up to 2014 (4). The $bla_{\text{CTX-M-27}}$ gene is, however, a more common finding in human health care (4, 45). In contrast, *E. coli* with the gene $bla_{\text{CMY-2}}$ is the single most common type found in clinical infections in dogs in Sweden comprising 34% of confirmed ESCRE isolates up to 2014 (4), but only 5–6% of human cases (4, 45). Moreover, *E. coli* with the gene $bla_{\text{CMY-2}}$ are commonly found in raw dog food diets that accordingly could be a source of such bacteria for dogs (46). However, the impact on carriage of such bacteria by dogs is not known.

In our study, only children below 3 years of age were colonized with the same ESCRE as the household dog during the 3-month study period. This suggests that toddlers and dogs of the same household have closer contact and thus may transfer bacteria between each other. Children are less aware of the hygiene aspects of living close to a dog, and in both household 1 and 2, the toddlers petted, kissed, and shared food with the dogs. This could facilitate spreading of ESCRE between dogs and persons in a household.

The major shortcoming of the present study is the small number of households included; thus, no statistical evaluation was possible. Future studies need to include larger number of households in order to make statistically reliable analysis. Another shortcoming is that dogs were sampled only on one occasion, which could have underestimated the extent of ESCRE carriage. Also, a part of the control group was recruited from another area (Uppsala) than the study group. This could have led to an underestimation of ESCRE carriage in dogs in households without persons carrying ESCRE. However, given the conceivably low carriage rate of ESCRE in healthy dogs (23), it is unlikely that this influenced the conclusions of the study. Also, for practical reasons different methods were used to screen human and dog samples for ESCRE. Both methods are well established and entail selective culture for ESBL- and pAmpC-producing Enterobacteriaceae, but the relative sensitivity of the two methods was not evaluated. Tentatively, a lower sensitivity of the method used in samples from dogs could result in an underestimation of the extent of sharing of strains.

In conclusion, this study shows that in households where humans are carrying ESCRE, identical strains were to a limited extent found also in household dogs, indicating transfer between humans and dogs. In contrast, ESCRE was not found in dogs in households without human carriers. The objective of this study was to study the sharing of ESCRE in households where humans were the identified carriers, focussing on transfer from human to dogs. It would, however, be equally relevant to study sharing of ESCRE, where dogs are the primary identified carriers of ESCRE.

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Conflict of interest and funding

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